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Huawei Pharmaceutical Com. Ltd.

Experiment Report of New Drug Screening

Study of the Compounds from YuePeng Gao Research Team of
Shi He Si Medical university

Director	Hongbing Zhao Ph. D.
Phone	15821697315
Time	2026.1.5-2026.1.12
Document	Huawei Pharmaceutical Com.
Website	www.huaweipharma.cn

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Derector: Hongbing Zhao

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1. Materials and methods

1.1. Compounds

1.1.1 compounds: see Table 1

1.1.2 LOT:

1.1.3 purity:

1.1.4 pack: 1.5 ml tube

1.1.5 M.W. and weight: see Table 1

1.1.6 Solution preparation: using DMSO

1.1.7 storage: -20°C

1.1.8 validity: 2015-12

1.1.9 provider: Study of the Compounds from YuePeng Gao Research Team of Shi He Si Medical university

Compound I.D.	Stock Concentration (mM)	Solvent
AH-262/34337058	10	DMSO
AN-829/14136109	10	DMSO
AO-476/40829168	10	DMSO
AF-399/40702931	10	DMSO
AF-399/15128462	10	DMSO
AE-848/32308062	10	DMSO
AK-968/40641331	10	DMSO
AI-204/31700025	10	DMSO
AR-471/43118674	10	DMSO
AO-476/43380499	10	DMSO
AO-476/43380403	10	DMSO
AG-205/12041012	10	DMSO
AG-690/36472028	10	DMSO
AI-346/32221059	10	DMSO
AG-690/11203385	10	DMSO
AE-842/34029016	10	DMSO
AF-399/42018988	10	DMSO
AH-262/36948011	10	DMSO
AE-848/30538026	10	DMSO
Gefitinib	10	DMSO

1.2. Reference compounds

- 1.2.1 name:
- 1.2.2 catalog:
- 1.2.3 purity: >98%
- 1.2.4 character:
- 1.2.5 validity: 2015-12
- 1.2.6 storage: -20°C
- 1.2.7 provider: Selleck Chemicals Co.,Ltd

1.3. Solvent

- 1.3.1 name: DMSO
- 1.3.2 catalog: D100627
- 1.3.3 specification: 500 mL
- 1.3.4 character: transparent liquid;
- 1.3.5 storage: room temperature
- 1.3.6 validity: 2015-12
- 1.3.7 provider: Sangon Biotech Co.,Ltd

1.4. Dosage designation

According to the literature

1.5. Methods

Theses assays were carried out as described previously.[01] All of the enzymatic reactions were conducted at 30°C for 40 minutes. The 50µl reaction mixture contains 40 mM Tris, pH 7.4, 10 mM MgCl₂, 0.1 mg/ml BSA, 1 mM DTT, 10 µM ATP, Kinase and the enzyme substrate. The compounds were diluted in 10% DMSO and 5µl of the dilution was added to a 50µl reaction so that the final concentration of DMSO is 1% in all of reactions. The assay was performed using Kinase-Glo Plus luminescence kinase assay kit. It measures kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The luminescent signal from the assay is correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity. The IC₅₀ values were calculated using nonlinear regression with normalized dose–response fit using Prism GraphPad software.

[01] Kashem, M.A.et al . (2006) Three mechanistically distinct kinase assays compared: Measurement of intrinsic ATPase activity identified the most comprehensive set of ITK inhibitors. J. Biomol. Screen . 12, 70–83.

Assay	Enzyme Used/ Reaction	Substrate
EGFR(WT)	25ng	0.2 mg/ml Poly (Glu, Tyr)/ 10 u M ATP

1.6. Quality control

All the experimental results will be considered to be credible when reference compound's result is similar as the literature result

1.7. Statistical

2. Results

2.1. Enzyme EGFR inhibition results table

Inhibition % = 100 - Activity %

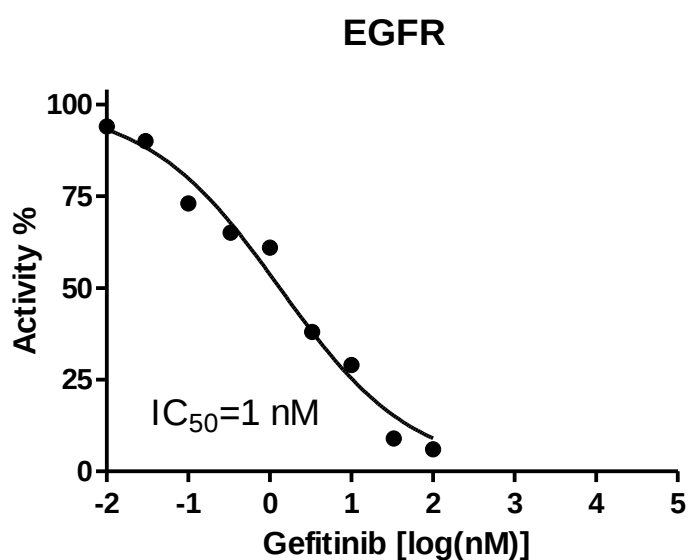
"Compound (@ 10uM)"	Inhibition %
AH-262/34337058	92
AN-829/14136109	97
AO-476/40829168	28
AF-399/40702931	95
AF-399/15128462	74
AE-848/32308062	95
AK-968/40641331	96
AI-204/31700025	97
AR-471/43118674	84
AO-476/43380499	94
AO-476/43380403	91
AG-205/12041012	92
AG-690/36472028	16
AI-346/32221059	81
AG-690/11203385	91
AE-842/34029016	83
AF-399/42018988	83
AH-262/36948011	72
AE-848/30538026	77
Gefitinib	IC ₅₀ = 2 nM

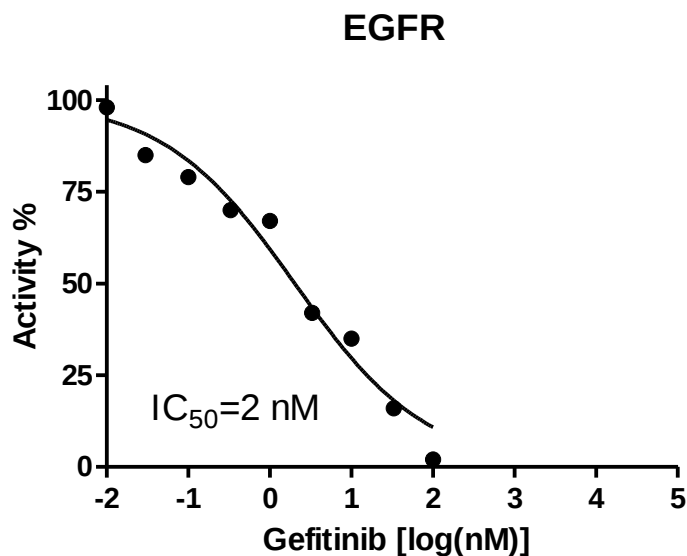
2.2. Compound result of EGFR enzyme activity influence

"Compound (@ 10uM)"	EGFR		Activity %	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	24431	23660		
AH-262/34337058	40405	39538	6	11
AN-829/14136109	41198	40699	1	4
AO-476/40829168	28383	29589	75	68
AF-399/40702931	41021	40263	2	7
AF-399/15128462	36495	37412	28	23
AE-848/32308062	40892	40126	3	8
AK-968/40641331	41159	40167	2	7
AI-204/31700025	41350	40490	1	5
AR-471/43118674	38215	39172	19	13
AO-476/43380499	40802	40091	4	8
AO-476/43380403	40255	39430	7	12
AG-205/12041012	40322	39733	6	10
AG-690/36472028	26224	27308	87	81
AI-346/32221059	37754	38636	21	16
AG-690/11203385	40456	39302	6	12
AE-842/34029016	37839	39084	21	14
AF-399/42018988	37883	39015	20	14
AH-262/36948011	35975	37050	31	25
AE-848/30538026	37002	38046	26	20
Background	42305	40584		

2.4. Compound Gefitinib result of EGFR enzyme activity influence

Gefitinib	EGFR		Activity %	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	24431	23660		
0.01 nM	25166	24313	94	98
0.03 nM	25822	26602	90	85
0.1 nM	28715	27737	73	79
0.33 nM	30067	29249	65	70
1 nM	30756	29723	61	67
3.3 nM	34858	34084	38	42
10 nM	36478	35396	29	35
33 nM	39900	38688	9	16
100 nM	40427	41030	6	2
Background	42305	40584		





3. Conclusion

4. Appendix

4. 1. Experimental data document

All experimental records are kept in HuaWei Pharmaceutical Com. archives room, including paper documents and electronic document; By default, the original document data will be saved about ten years in my company.

4.2. Compounds storage

All the experimental samples were stored in the HuaWei Pharmaceutical Com. sample library, and storage conditions is - 20 °C avoiding light. The default time of saving the samples is one year, and samples will be disposed one year later, in order to protect the customer's project secret.